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Avian Synaptopodin 2 (Fesselin) Inhibits Actomyosin Dissociation by ATP and Alters the Structure of Smooth Muscle Myosin Filaments

Nathaniel Kingsbury[1], Randall Renegar[2], Mechthild M. Schroeter[3], Joseph M. Chalovich[1].
[1]Biochemistry & Molecular Biology, Brody School of Medicine at East Carolina University, Greenville, NC, USA, [2]Anatomy & Cell Biology, Brody School of Medicine at East Carolina University, Greenville, NC, USA, [3]Institut für Vegetative Pysiologie, University of Cologne, Germany.

Fesselin or avian synaptopodin 2 stimulates actin polymerization in a Ca^{2+}-calmodulin dependent manner. Fesselin binding to F-actin inhibits myosin S1 binding and yet fesselin binds with moderate affinity to smooth muscle myosin. These properties suggest that fesselin could tether actin and myosin together in an inactive complex as caldesmon does. This possibility was tested by observing the effect of fesselin on the rate of dissociation of actin-myosin by ATP in a stopped-flow device. Dissociation was measured by light scattering (a measure of particle size) and by pyrene actin fluorescence (a specific measure of actin-myosin binding). Fesselin reduced the multi-exponential rates of change of light scattering and pyrene fluorescence in concentration dependent manners. Each light scattering trace had a rapid initial transition that was not present in pyrene fluorescence traces. That rapid light scattering change was likely due to dissociation of filamentous myosin. The reduction in rate of that rapid process could mean that fesselin alters the structure and dissociation kinetics of smooth myosin filaments. We examined changes in smooth muscle myosin with electron microscopy. ATP caused dissociation of myosin filaments in both the presence and absence of fesselin. In the absence of ATP, fesselin increased the size of myosin filaments. Furthermore, the filaments were arranged in parallel arrays. These results indicate that fesselin cross-links myosin to actin and also organizes myosin filaments in solution.

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Vimentin Filament Assembly is Altered by Substrate Elastic Modulus

Melissa G. Mendez, Maria E. Murray, David J. Restle, Paul A. Janmey.
University of Pennsylvania, Philadelphia, PA, USA.

Background: The cytoskeletal protein vimentin is involved in the determination of cell mechanical properties, shape and motile behavior. We are investigating how vimentin participates in the response to external mechanical cues.
Methods: Normal and mouse embryo fibroblasts (mEF) harvested from the vimentin-knockout mouse are grown on fibronectin-coated polyacrylamide gels (Fn-PAA) of 0.5-40 kPa. Protein expression levels are assayed by Western blotting, and vimentin network distribution by immunofluorescence. Rates of vimentin subunit turnover are assayed by fluorescence recovery after photobleaching (FRAP) experiments; and atomic force microscopy (AFM) is used to measure cells' elastic and viscoelastic-plastic properties.
Results: Vimentin protein expression levels do not change in response to substrate stiffness. Whereas FRAP results in other cell types - which are less phenotypically responsive to substrate stiffness - show no difference in the rate of vimentin subunit turnover across various substrates, early results suggest that subunit turnover increases when fibroblasts are grown on physiological-range stiffnesses (~6 kPa). Also, many short vimentin filaments and squiggles (<15 μm in length) are evident that appear not to be connected to the extended vimentin network, and vimentin is more detergent-soluble under these conditions. The responses (e.g. shape, motility, etc.) of vimentin-null fibroblasts confirm vimentin's participation in these processes across the range of substrate stiffnesses. Finally, vimentin-null mEF are less stiff than mEF on ~6 kPa Fn-PAA, and initial experiments show that whether vimentin makes cells softer or stiffer depends upon the substrate elastic modulus.
Conclusion: Vimentin is expressed at consistent levels across conditions that cause changes in cell stiffness, shape and motility, yet the response of vimentin-knockout cells to the same conditions demonstrates vimentin's role in response to mechanical stimuli. We hypothesize that modulation of vimentin's assembly state underlies its contribution to cell mechanics.
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Actin Dynamics within Single Dendritic Spine Investigated by Two Photon Fluorescence Correlation Spectroscopy during Synaptic Plasticity

Jian-Hua Chen[1], Yves Kellner[2], Marta Zagrebelsky[2], Matthias Grunwald[1], Martin Korte[2], Peter Jomo Walla[1,3].
[1]Max-Planck Institute for Biophysical Chemistry, Goettingen, Germany, [2]Division of Cellular Neurobiology, Zoological Institute, TU Braunschweig, Braunschweig, Germany, [3]Department of Biophysical Chemistry, Institute for Physical and Theoretical Chemistry, TU Braunschweig, Braunschweig, Germany.

Dendritic spines are the major site of excitatory synaptic input to pyramidal neurons of the hippocampus. The activity-dependent changes in the structure of spine structure have been clearly related to learning and memory processes. Actin is known to regulate cell shape and motility and in mature neurons is highly concentrated within dendritic spines (1). Indeed, the integrity as well as the changes in the spine structure have been shown to depend on the actin cytoskeleton dynamics. However, how actin filament dynamics are regulated during activity-dependent structural changes at synapses is up to now largely unexplored. Our study, by combining fluorescence microscopy (FM) with two-photon fluorescence correlation spectroscopy (2P-FCS)(2), allows us to simultaneously monitor the morphological changes and the dynamic behavior of actin filaments within single dendritic spine of neurons expressing actin-eGFP before and after chemical induction of synaptic plasticity (cLTP). Analyzing the autocorrelation curves from the 2P-FCS measurements and fitting them with two components diffusion model provide us quantitatively results, which show that: (1) actin dynamics within spines are significantly altered upon C-LTP induced morphological changes, (2) the highly dynamic actin filament exhibit a. heterogeneous structural composition, and (3) the regulations of actin filaments in single dendritic spine are precisely controlled individually instead of being a globally homogeneous function.
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Microfluidics with In-Situ Small-Angle X-Ray Scattering: A Tool to Investigate the Neurofilament Self-Assembly Mechanism

Bruno F.B. Silva[1,2], Miguel Z. Rosales[3], Joanna Deek[4], Ulf Olsson[2], Youli Li[3], Cyrus R. Safinya[1].
[1]Department of Physics, Department of Materials, and Molecular, Cellular & Developmental Biology Department, University of California Santa Barbara, Santa Barbara, CA, USA, [2]Division of Physical Chemistry, Centre for Chemistry and Chemical Engineering, Lund University, Lund, Sweden, [3]Materials Research Laboratory, University of California, Santa Barbara, Santa Barbara, CA, USA, [4]Chemistry and Biochemistry Department, University of California, Santa Barbara, Santa Barbara, CA, USA.

The use of microfluidic chips with in-situ small-angle X-ray scattering (SAXS), offers new interesting possibilities for the study of biomaterials under flow. In first place, the manipulation of fluids allows for an experimental control (e.g. rate of mixing, shear rate, concentration gradients, confinement) that has been previously unavailable, opening the possibility for new experiments. In second place, sample consumption is reduced to the microliter scale, allowing experiments with expensive and rare materials. In third place, the constant flow of material prevents radiation damage (critical for X-ray synchrotron radiation).
In this work, we describe an approach to probe the mechanism of self-assembly of mature neurofilaments from its individual protein subunits at physiological ratio (NF-L:NF-M:NF-H of 7:3:2). This process is believed to consist of a series of distinct steps in vitro, involving the formation of several intermediate structures, but its full description has not been reported so far (Janmey PA. et al., 2003, Curr. Opin. Colloid Interface Sci. 8:40-47).
The protein solution under strong denaturing conditions (4 and 8 M urea, which prevents the assembly process) is mixed under flow with physiological buffer, leading to a drop in the urea concentration to levels where filament formation is favored. The geometry of the chip allows time-resolved tracking of the assembly process along the main microchannel. The emergence of new structural features with time, typical of larger aggregates, compared to the initial unimer solutions, is observed.
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Salt-Responsive Liquid Crystal Hydrogels: Neurofilament Network Structure and Mechanical Modulation

Joanna Deek[1], Peter J. Chung[1], Jona Kayser[2], Prof. Andreas Bausch[2], Prof. Cyrus R. Safinya[1].
[1]University of California, SB, Santa Barbara, CA, USA, [2]Technische Universität München, Garching, Germany.

Neurofilaments (NFs), the class of intermediate filaments found in neurons, are one of three structural protein groups that collectively form the cytoskeletal network. The active structure that NFs assume in the cell, and that permits

them to fulfill their adaptable physiological role, is a space-filling expanded hydrogel held together by tunable interfilament associations. These interfilament associations are predominantly electrostatic and established by the polyampholytic nature of the sidearms, the unstructured C-termini of the three constituent subunits: NF-Low (NF-L), NF-Medium (NF-M), and NF-High (NF-H) [1]. Using synchrotron x-ray scattering and polarized optical microscopy, we examined the strength of these regulatory electrostatic associations and their effect on filament re-orientation organization. As a function of decreasing ionic strength, for binary and ternary NF systems, three distinct salt-induced hydrogel phases are found: nematic liquid-crystal (NG), isotropic (IG), and anisotropic NF blue phase (BG). At low ionic strength (< 5mM) with weak screening, sidearm overlap is maximal producing BG hydrogels with very high elastic moduli comparable to cross-linked gels with a phenomenal ability to retain shape and water over long-times. Upon melting into the IG phase, with reduced sidearm overlap, filaments re-organize crossing at large angles to minimize electrostatic repulsion. At still higher salt concentrations, NF gels exhibit an abrupt transition from the IG with mesh size $\approx$ 1000Å to an Onsager-type oriented nematic liquid-crystalline gel with interfilament spacing d $\approx$ 500Å (NF-LM) and 700Å (NF-LH)[1]. Remarkable to the NF system is the fully reversible interchange of NF hydrogel properties, most notably the elastic modulus transition from chemical-gel mimicking BG to physical-gel-like (IG and NG), prompted merely by variation in ionic strength. Supported by DOE-BES DOE-DE-FG02-06ER46314 and NSF DMR-1101900.

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Shear-Optimized Platelet-Like-Particles from High Ploidy MKS: From Segregation to Composition and Activation

Kyle Spinler, Jae-Won Shin, Dennis E. Discher.
University of Pennsylvania, Philadelphia, PA, USA.

Cone and plate rheometry is used to impart physiologically relevant shear stress on the human megakaryocyte (MK) cell line, MEG01. These experiments reveal that platelet-like-particle (PLP) generation is sensitive to both biomechanical and pharmacological factors namely blebbistatin inhibition of NMM-II. We demonstrate that shear stress reduces phospho-deactivation of NMM-II heavy chain at Ser1943 to approximately 30% of the unsheared level, indicating a restoration of NMM-II activity necessary for proper platelet function. Stimulation of rheometer generated PLP cultures with collagen-I showed aggregation and phosphatidylserine exposure (with Annexin-V binding in the presence of Ca^{2+}). These data demonstrates that PLPs generated in this system retain some degree of functionality such that MKs exposed to shear stress and blebbistatin result in approximately 6.5 fold more PLPs than untreated MK cultures.

To assess partitioning and segregation of proteins from MKs in sheared membranes, we used fluorescence-imaged micro-deformation (FIMD) to monitor CD41 and NMM-IIA during micropipette aspiration. Antibody labeling of MK surface CD41 shows a homogeneous intensity along the aspirated projection of membrane, but cell body shows approximately 3 fold higher intensity, suggesting an excess of CD41 in the MK. Pre-treatment with blebbistatin increases fragmentation frequency, and these fragments show a similar trend with CD41 expression. Nucleofection was used to introduce either GFP tagged WT NMM-IIA or phosphomemetic, myosin deactivating, GFP tagged NMM-IIA S1943D to assess whether pSer impacts partitioning of this cytoskeletal protein that is abundant in platelets. Both WT and S1943D NMM-IIA are seen in the aspirated cell projection. WT NMM-IIA accumulates at the leading edge of the aspirated projection and at sites of membrane fragmentation, whereas S1943D remains uniformly dispersed. These findings underscore the central role of NMM-II heavy chain phosphorylation, and thus activity, in proplatelet formation and platelet fragmentation.

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Mechanics and Structure of Fibrin Networks Polymerized under Oscillatory Shear Perturbations

Stefan Münster[1,2], Louise M. Jawerth[2], David A. Weitz[2], Ben Fabry[1].
[1]FAU Erlangen-Nuremberg, Erlangen, Germany, [2]Harvard University, Cambridge, MA, USA.

The mechanical properties of a blood clot are of crucial importance for its ability to stem the flow of blood at a site of vascular injury. These properties are largely determined by the underlying structural scaffold which forms during blood coagulation, a branched network of the biopolymer fibrin. Alterations in the structure of this network, such as changes in fiber density, fiber thickness or branching probability, strongly affect the mechanics of the network. The relationship between network architecture and bulk-level mechanics is commonly investigated using in vitro fibrin networks polymerized under static conditions. However, in the body, blood clots form under a highly dynamic mechanical environment: Nascent fibrin fibers are constantly exposed to the pulsatile shear flow of blood and the concurrent oscillatory dilation of the vessel walls. However, the effects of mechanical perturbations during polymerization on clot structure and the resultant mechanical properties remain unknown. Here, we polymerize fibrin networks while applying continuous oscillatory shear perturbations of varying strain amplitude. Despite these mechanical perturbations, fibrin can form rigid clots which exhibit a significantly later onset of the non-linear strain-stiffening response, a postponed rupture strain, and a lowered linear modulus compared to clots formed without perturbations. Up to perturbation amplitudes of 45% shear strain, the typical non-linear stiffness of these clots as well as their rupture stresses are of similar magnitude to those formed without perturbation. We show by confocal microscopy that these changes in the mechanical properties result from a formation of two architecturally distinct layers within the clot: one layer shows a highly bundled structure, while the other layer is virtually unaltered. This architectural adjustment may serve as a means for adapting blood clots to the mechanical loading conditions of the environment in which they form.

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Superresolution Investigation of the Dynamics of FtsZ Structures during E. Coli Cell Division

Carla Coltharp, Jackson Buss, Jie Xiao.
JHU, Baltimore, MD, USA.

FtsZ is an E. coli tubulin homolog that forms single-stranded protofilaments that can interact laterally to form superstructures. In vivo, FtsZ localizes to the midcell plane and assembles into a ring-like structure known as the Z-ring, which is necessary for cell division. The Z-ring serves as an scaffold to recruit all other division proteins and may also generate contractile force for cytokinesis. However, details about the arrangement of protofilaments within the Z-ring, which would suggest possible contraction mechanisms, remains unclear. We have used photoactivated localization microscopy (PALM) to characterize the in vivo structural dynamics of the Z-ring in E. coli at a spatial resolution of ~35 nm. Using Photoactivated Localization Microscopy (PALM), we have previously shown that the Z-ring is a loose bundle of overlapping protofilaments that form either a single-ring conformation at midcell or a multiple-ring conformation reminiscent of a tight helix. We are currently investigating the structural changes that the Z-ring undergoes during the cell cycle and the role of GTPase activity in the structural rearrangements using the variant FtsZ84, which has diminished GTPase activity in vitro.

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Metamorphic Pattern Formation and Deformation: In Vivo and In Vitro Mechanisms

Zachary T. Olmsted, Tim D. Riehlman, Carmen Branca, Andrew G. Colliver, Adam M. Winnie, **Janet L. Paluh**.
College of Nanoscale Science & Engineering, UAlbany-SUNY, Albany, NY, USA.

Mitotic spindle formation into a bipolar structure suitable for chromosome segregation requires reorganization of the interphase microtubule cytoskeleton. A multi-protein complex at spindle poles acts as a microtubule organizing center (MTOC). Microtubules are assembled from this site through the addition of α/β-tubulin heterodimers onto a template complex containing γ-tubulin (γ-TuSC) imbedded into a larger macromolecular ring (γ-TuRC). Our goal is to apply insights on patterning biological polymers in vivo to development of hybrid biosynthetic systems capable of utilizing microtubules in self-assembling metamorphic patterns including parquet deformation behavior. Dynamic patterning has applications in biosensing, materials design and new nanomanufacturing paradigms. Building off of recent structural insights into γ-TuSC and GCP4 with our own detailed genetic analysis, site-directed mutagenesis, cross-species functional studies and biochemical purification and nucleation assays we provide novel insights on MTOC structural requirements to nucleation. Additionally we have identified an associated regulatory mechanism utilized by a subset of Kinesin-14 members for targeting and regulatory interference at poles (TRIP) distinct from microtubule targeting elements found in other Kinesin-14 members such as *Drosophila* Ncd. Our in vivo analysis supports application of the recent Kollman-Agard structure as a general eukaryotic model however with species-specific protein and domain constraints as well as contact sites for Kinesin-14 regulation of γ-TuRC. Our findings have broad application towards a general understanding of cellular MTOC machinery and reiterates the flexibility of Klps to localize to multiple spindle